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BEFORE THE BOARD OF PATENT APPEALS AND INTERFERENCES

Application Number: 10/685,737
Filing Date: October 15, 2003
Appellant(s): RUBIN ET AL.

Isadora F. Bielsky
<u>For Appellant</u>

EXAMINER'S ANSWER

This is a supplemental Examiner's Answer in response to the appeal brief filed 12/04/2008 appealing from the Office action mailed 6/10/2008. The rejection under 35 USC 101 set forth in the Examiner's Answer of 3/19/2009 has been withdrawn; no new grounds of rejection are cited herein.

(1) Real Party in Interest

A statement identifying by name the real party in interest is contained in the brief.

(2) Related Appeals and Interferences

The examiner is not aware of any related appeals, interferences, or judicial proceedings which will directly affect or be directly affected by or have a bearing on the Board's decision in the pending appeal.

(3) Status of Claims

The statement of the status of claims contained in the brief is correct.

(4) Status of Amendments After Final

No amendment after final has been filed.

(5) Summary of Claimed Subject Matter

The summary of claimed subject matter contained in the brief is correct.

(6) Grounds of Rejection to be Reviewed on Appeal

The appellant's statement of the grounds of rejection to be reviewed on appeal is correct.

(7) Claims Appendix

The copy of the appealed claims in the replacement section filed on 8/19/2009 of the Appendix to the brief is correct.

(8) Evidence Relied Upon

Marks et al., US Patent 6,794,128

Dunlay et al., US Patent 5,989,835

Kallal et al., “Visualization of Agonist-induced Sequestration and Down-regulation of a Green Fluorescent Protein-tagged-beta-2-Adreneric Receptor." Journal of Biochemistry, vol. 273 (1998) pages 322-328.

Proffitt et al., “A Fluorescence Digital Image Microscopy System for Quantifying Relative Cell Numbers in Tissue Culture Plates,” Cytometry, vol. 24 (1996) pages 204-213.

(9) Grounds of Rejection

The following ground(s) of rejection are applicable to the appealed claims:

1. The following is a quotation of 35 U.S.C. 103(a) which forms the basis for all obviousness rejections set forth in this Office action:

> (a) A patent may not be obtained though the invention is not identically disclosed or described as set forth in section 102 of this title, if the differences between the subject matter sought to be patented and the prior art are such that the subject matter as a whole would have been obvious at the time the invention was made to a person having ordinary skill in the art to which said subject matter pertains. Patentability shall not be negatived by the manner in which the invention was made.

2. This application currently names joint inventors. In considering patentability of the claims under 35 U.S.C. 103(a), the examiner presumes that the subject matter of the various claims was commonly owned at the time any inventions covered therein were made absent any

evidence to the contrary. Applicant is advised of the obligation under 37 CFR 1.56 to point out the inventor and invention dates of each claim that was not commonly owned at the time a later invention was made in order for the examiner to consider the applicability of 35 U.S.C. 103(c) and potential 35 U.S.C. 102(e), (f) or (g) prior art under 35 U.S.C. 103(a).

Claim 40-42 and 44-48 are rejected under 35 U.S.C. 103(a) as being unpatentable over Marks et al. (US Patent 6,794,128) in view of Kallal et al. (Journal of Biochemistry, vol. 273 (1998) pages 32-328) in further view of Proffitt et al. (Cytometery, vol. 24 (1996) pages 204-213).

The instant claims are drawn to a machine readable storage medium comprising a program containing instructions for measuring internalization of cell surface proteins through a cell screening system.

1. Claim 40 recites identifying internalized cell surface receptor proteins in cells where individual cells comprise at least a first luminescent reporter molecule that reports on the cell surface receptor and at least a second luminescent reporter molecule that reports on cells. Internalized cell surface receptors are identified by determining a luminescent signal from the first reporter molecule that surpasses a user defined intensity.

2. Marks et al. teach a method of internalizing phages into target cells and identifying the internalized phages (Abstract). The prior art teaches a method of identifying internalizing antibodies and internalizing receptor ligands (col. 1, lines 20-26, col. 3, lines 17-25). This includes identifying internalizing antibodies as well as internalizing receptors (col. 1, lines 20-25). The method can be carried out by labeling the phage with a reporter gene encoding a

fluorescent protein such as GFP or a luciferase (col. 2, line 66 to col. 3, line 5; and col. 4, lines 9-12).

3. Marks et al. teaches a method of identifying internalized receptors (col. 13, lines 44-55) as well as using reporter genes to identify cells that express GFP. This method can be used to identify target cells (col. 17, line 45 to col. 18, line 22) within a subtractive cell line (col. 18, lines 23-65). Here, the subtractive cells display all the markers of the target cell except the marker (e.g. receptor) that is to act as a target for the desired binding of antibodies or binding polypeptides. This reads on the limitations set forth in claim 40(a) where each cell is contacted with at least two reporter molecules and claim 47 reciting a second reporter molecule that is a fluorescent reporter molecule.

4. Furthermore, Marks et al. teach the labeling of antibodies with fluorescent labels that in turn report on the internalization of receptors (col. 9, lines 7-37 and col. 12, line 40 to col. 13, line 55), as in claim 45.

5. Marks et al. goes on to teach identification of an internalized phage (col. 19, line 57 to col. 20, line 10) with the use of a detectable fluorescent signal where the phage bears a marker (e.g. label) and the surface bound or internalized phages are sorted (col. 20, lines 3-14).

6. The prior art of Marks et al. teaches the measuring of internalized phages with FACS (fluorescence activated cell sorting) (col. 46, lines 47-48; col. 47, line 65 to col. 48, line 3; and Figure 9). This reads on the limitations set forth in claim 40(b) where calculations on cells that have internalized the luminescently labeled reporter molecule are performed.

7. Claim 40, step (c) recites displaying data on internalized cell surface receptor proteins.

8. Marks et al. teaches a table containing data on cell surface bound phage and internalized phage (col. 29, Table 4).

9. Claim 41 recites the steps (a) and (b) carried out at multiple time points. As illustrated in Figure 9, the internalization of the phages is calculated at multiple time points.

10. Claim 42 recites determining an aggregate area of the objects that represent the internalized cell surface receptor protein (step (i)) and a number of objects that represent the internalized cell surface receptor protein (step (iv)).

11. Marks teaches that the methods of the prior art invention can be used to identify internalizing receptors and regions of the receptor that when bound induce internalization of the binding moiety (col. 3, lines 17-20), as in claim 41, step (i). Marks further teaches the identifying of internalized members of the phage display library if the members are internalized into one or more of the target cells (col. 3, lines 38-40).

12. Marks does not specifically teach a reporter molecule that labels a cell surface receptor protein to produce a labeled cell surface receptor protein or that the luminescent signals are from the "labeled cell surface receptor protein" and luminescent signals are from the "labeled cell surface receptor protein", as recited in claim 40, step (a). Marks also does not teach a first luminescent reporter molecule that comprises a fluorescent protein, fluorescent reporter molecule, or a cell surface receptor protein which is a G-protein coupled receptor, as in claims 44, 46 and 48 respectively.

13. Kallal et al. teach the direct labeling of the β_2-Adrenergic Receptor (β_2AR) with a green fluorescent protein (as required in claims 44, 46 and 48) to study the β_2AR trafficking including internalization of β_2AR (Abstract; page 322, col. 1, ¶1). Agonist-mediated internalization of

β_2AR-GFP was assessed (page 324, col. 1, ¶2). Kallal et al. teach the study of internalization of β_2AR-GFP wherein fluorescent images are obtained from single cells (page 324, col. 2, ¶ 2 to page 325, col. 1) (i.e. measure of internalization), as in claim 40(a).

14. Marks in view of Kallal et al. does not teach calculating a number and or percent of the individual cells that internalized the at least first luminescently labeled reporter molecule. Marks in view of Kallal et al. also do not teach a machine readable storage medium comprising a program that executes procedures for measuring internalization of cell surface receptor proteins.

15. Proffitt et al. however teaches a computerized scanning system and algorithm (page 207, col. 1, ¶ 3) that is able to measure the relative cell numbers (page 204, col. 1, lines 1-4) that contain a fluorescent label. The total relative fluorescence intensity for the entire well containing cells is determined, "which is proportional to cell number" (page 207, col. 1, ¶4 to col. 2, ¶ 1). Figure 3 shows the number of "Cells per Well" that can be used to calculate the number of cells with respect to the "Relative Fluorescence". The method of Proffitt et al. in Figure 3 for measuring fluorescence for a quanitifed number of cells renders the obverse method obvious; i.e. to determine the number of cells at a value of relative fluorescence.

16. It would have been prima facie obvious to one of ordinary skill in the art at the time the invention was made to modify the procedure of labeling antibodies and bacteriophages that are internalized by a cell with a fluorescent proteins as taught by Marks with the method of Kallal et al. that directly labels the receptor with GFP. One of skill in the art would have been motivated to label the receptor protein directly because Kallal et al. teach that this technique provides for a more extensive optical analysis of the internalization and recycling of the receptor (Abstract). One of skill in the art would have had a reasonable expectation of success at utilizing the method

of Marks with that of Kallal et al. because both teach the study of internalizing receptors using fluorescence.

17. It would have been prima facie obvious to one of ordinary skill in the art at the time the invention was made to modify the procedure of labeling the internalizing receptor with fluorescent protein as taught by Marks in view of Kallal et al. and then measuring the number of cells that had the internalized fluorescent protein with the computerized scanning system as taught by Proffitt et al. One of skill in the art would have been motivated to use the cell quantifying fluorescently labeled cells as taught by Proffitt et al. to measure the internalization of cell surface receptors as taught by Marks in view of Kallal et al. because Proffitt et al. teaches that this is an effective method of determining which cells are viable cells (page 211, col. 1, ¶1). One of skill in the art would have had a reasonable expectation of success at utilizing the computerized scanning system that determines the number of fluorescing cells taught by Proffitt et al. with the assays of internalized reporter molecules as taught by Marks because Marks and Kallal et al. teach labeling using fluorescent proteins and wherein the system of Proffitt et al. measures fluorescence.

3. Claim 43 is rejected under 35 U.S.C. 103(a) as being unpatentable over Marks in view Kallal et al. in view of Proffitt et al. as applied to claims 40-42 and 44-48 above, and further in view of Dunlay et al. (US Patent 5,989,835, in IDS filed 5/19/04).

4. Marks et al. in view of Kallal and Proffitt et al. make obvious the identification of internalized receptors wherein the cells contain internalized green fluorescent proteins that report on cells that contain the internalized receptors and wherein Proffitt et al. teach a computerized

scanning system that scans and quantifies the fluorescence in cells to determine the number of cells. Internalizing antibodies in an affinity matrix or solid support are taught by Marks (col. 13, lines 56-62) as required by claims 40-42. However, Mark, Kallal and Proffitt et al. do not teach images of the array to obtain both low and high resolution images of those array locations that contain internalized cell surface receptor proteins.

5. Dunlay et al. teach providing cells containing fluorescent reporter molecules in an array of locations and scanning numerous cells in each location with a fluorescent microscope (Abstract). The whole area of the plate can be imaged (col. 1, lines 32-37) where cells have been treated with fluorescent reagents such as GFP and expressing GFP in cells for use as reporter molecules (col. 2, lines 11-53). Further Dunlay et al. teach imaging the array of cells at a low resolution and imaging particular locations in the microplate at a higher resolution (col. 5, lines 19-27).

6. It would have been prima facie obvious to one of ordinary skill in the art at the time the invention was made to have implemented the imaging technique of Dunlay et al. to image the cells with internalized GFP reporter molecules that report on internalized receptors as taught by Marks, Kallal et al. and Proffitt et al. One of skill in the art would have been motivated to use the multi-resolution imaging technique of Dunlay et al. in the method to be performed by the computer readable media-contained instructions of Marks, Kallal et al. and Proffitt et al. because Dunlay et al teach that using two resolutions improves the overall throughput of the screening system (col. 4, lines 25-27).

(10) Response to Argument

A. Summary of Appellant's Position

Appellants argue (Appeal Brief, page 5) the references alone or in combination do not teach calculating a number and/or percent of the individual cells that internalized the labeled cell surface receptor protein wherein the calculation provides a measure of internalization of the cell surface receptor protein in the individual cells.

B. Appellant's Arguments

Appellants argue (Appeal Brief, page 6) that Proffitt et al. does not teach "calculating a number and/or percent of the individual cells that internalized the at least first luminescently labeled reporter molecule". Instead, Proffitt et al. teach calculating the intensity of the entire well and measures the relative cell number. Therefore, Proffiet et al. does not teach measuring fluorescence from individual cells.

With respect to Appellants argument that Proffitt et al. does not teach "calculating a number and/or percent of the individual cells that internalized the at least first luminescently labeled reporter molecule," Proffitt et al. teaches that assay systems using fluorescent dyes are useful for "rapidly measuring cell numbers directly" (page 204, col. 2, ¶1). Proffitt et al. also teach that their digital image microscopy scanning system (DIM-SCAN) can quantify total or viable cell numbers in tissue culture plates using fluorescent dyes (page 205, col. 1, ¶1). In particular, Proffitt et al. teach that DIM-SCAN gives a linear proportion of relative fluorescence vs. the number of cells per well (Figure 3). To test this, Proffitt et al. produce a dilution series wherein the number of cells are known to be between 340 and 350,000 cells/well (page 208,

col. 1, ¶ 3) and measure the relative fluorescence, as shown in Figures 3 and 5. Proffitt et al. teach calculating 1,000 cells/well and determining “excellent linearity” with respect to fluorescence (page 208, col. 1, 2 lines from bottom). Using the data in Figures 3-4, it would be obvious for one of skill in the art to "calculate a number and/or percent of the individual cells" based on the known values of relative fluorescence.

Applicants argue that Proffitt et al. teaches “relative cell number” based on the intensity of fluorescence of the “entire well” and does not teach measuring fluorescence from individual cells (Brief, page 6, ¶1 to page 7 ¶3).

In response, instant claim 40, step (a) recites identifying internalized cell surface receptor proteins in multiple individual cells on the array of locations and in step (b), calculating a number and/or percent of the individual cells. The instant claim reads on calculating the number of the plurality of cells as taught by Proffitt et al. The instant claim does not require calculating the number and/or percent of cells based on a determination of **each** individual fluorescing cell in the assay. It would be obvious for one of skill in the art to determine the number of individual cells since the plurality of cells in the wells comprise individual cells. Using the Fluorescence vs. Cells per well data of Figures 3-5. Proffitt et al. teach calculating 1000 cells/well using the data in Figure 5 (page 208, col. 1, two lines from bottom) to determine "excellent linearity in 6, 24, and 48 well plates,” as given by the DIM-SCAN. Furthermore, though the instant study by Proffitt et al. is a test of the DIM-SCAN apparatus, Proffitt et al. teach that ultimately DIM-SCAN is used to “quantify total or viable cell numbers in tissue culture plates” (page 205, col. 1, ¶1). Therefore, the art of Proffitt et al. at least suggests "calculating the number and/or percent of individual cells," as recited in claim 40.

(11) Related Proceeding(s) Appendix

No decision rendered by a court or the Board is identified by the examiner in the Related Appeals and Interferences section of this examiner's answer.

For the above reasons, it is believed that the rejections should be sustained.

Respectfully submitted,

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